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EDGEWOOD ARSENAL TECHNICAL REPORT

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DDT-INDUCED REDUCTION IN EGGSHELL THICKNESS, WEIGHT, AND CALCIUM IS ACCOMPANIED BY INHIBITION OF CALCIUM ATPASE

by

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March 1977

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Prolonged feeding of DDT reduces activity of calcium-transporting ATPase in the microsomal fraction of eggshell gland mucosa. Concurrent findings of thin, calcium-deficient eggshells indicate that DDT produces thin-shelled eggs by impairment of calcium transport through the avian eggshell gland.

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PREFACE

The work described in this report was performed for the Environmental Protection Agency under the auspices of its toxicology contract with Edgewood Arsenal, EPA-IAG-D6-0429. This work was started in December 1975 and completed in April 1976.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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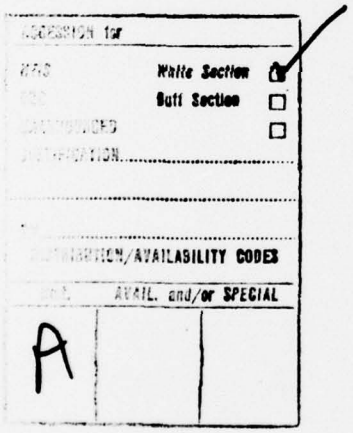

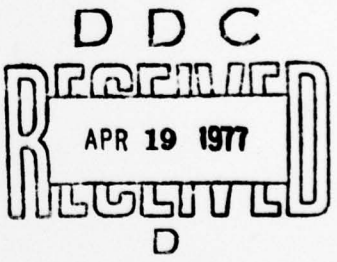

CONTENTS

DDT-INDUCED REDUCTION IN EGGSHELL THICKNESS, WEIGHT, AND CALCIUM IS ACCOMPANIED BY INHIBITION OF CALCIUM ATPASE

I. INTRODUCTION.

Environmental exposure to the pesticide 2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane (DDT) has been shown to cause thin-shelled eggs in a variety of avian species.[1-6] Further work has shown thin-shelled eggs to be deficient in calcium in spite of normal serum calcium levels.[7] Thus, it is likely that the defect in calcium transport occurs within the eggshell gland, that portion of the reproductive tract responsible for deposition of calcium into the forming eggshell.[8] A calcium-dependent ATPase has been found in high concentration in the microsomal fraction of the eggshell gland mucosa and is thought to be responsible for calcium transport.[9] One metabolite of DDT, namely, 2,2,bis-(chlorophenyl)-1,1-dichloroethylene (DDE), inhibited Ca ATPase in the homogenate of eggshell gland from Pekin ducks.[10]

II. MATERIALS AND METHODS.

Mature domestic mallard ducks (Anas platyrhynchos) were randomly assigned to two cages with five hens and one drake per cage. The ducks were acclimated to the housing area for 1 month, and during this time were fed poultry reproductive mash (Purina Chow) *ad libitum*. Upon initiation of the exposure, treated ducks were fed poultry mash containing 50 ppm DDT. Control ducks were fed the original poultry mash without DDT. Feeding was continued for 6 months. After 5 months of feeding, egg production was induced by increasing the photoperiod from 8 to 16 hours/day. Eggs were collected daily and eggshell weight, length, width, and thickness were determined. In order to correct for changes in eggshell thickness due to changes in size, a ratio of the weight/(length × width) (R-Value) was calculated.[11] After 6 months, the hen ducks were euthanatized by cervical dislocation.

The eggshell glands were rapidly excised and placed in ice-cold 0.25 M sucrose. The epithelium was removed by scraping with a clean razor blade and 1 gm was diluted to 10 ml in ice-cold 0.25 M sucrose. The mixture was homogenized in a Potter-Elvehjem tissue homogenizer at 400 rpm. The homogenate was centrifuged at 18,000 × g max in a Beckman L-2 ultracentrifuge at 4°C; the resulting supernatant was recentrifuged at 98,000 × g max and the pellet from this centrifugation was suspended in 0.25 M sucrose to a final volume of 3 ml. In order to assess the purity of the microsomal pellets, electron microscopic evaluation was performed. Aliquots of microsomal suspension were fixed in an equal volume of 8% glutaraldehyde in 0.4 M phosphate buffer at pH 7.2. Following sedimentation in a clinical centrifuge at 1,500 rpm for 1 minute, the pellets were processed for electron microscopic examination by routine methods.[12] The microsomal preparations were stored at -60°C until the time of assay.

Calcium ATPase activities were determined by the methods of Rorive and Kleinzeller.[13] The amount of phosphate liberated was determined by Stanton's[14] method. Calcium ATPase was expressed as μmoles Pi (inorganic phosphate) liberated/mg protein/30 minute incubation. Protein from the microsomal pellets was determined by the method of Lowry *et al.*[15]

Calcium was extracted by ashing a 0.5-gm sample of eggshell at 450°C for 12 hours. The ash was dissolved in concentrated HCl and calcium determinations were done by atomic absorption spectrophotometry.[16]

III. RESULTS.

When ducks were fed 50 ppm DDT for 6 months and brought into egg production, eggshell weights, thicknesses, and R-values were reduced (table 1). As shown, exposure to DDT resulted in eggshells which were reduced in thickness by 18% and in weight by 12%. Calculation of the R-values in both groups showed a reduction of 16% after exposure to DDT. Statistical analysis of length and width revealed no change in these parameters. When eggshells from control and DDT-fed ducks were analyzed for calcium content and weight, both parameters showed a reduction (table 2). Furthermore, the % reduction in both parameters was nearly the same (23% and 27%, respectively). It is interesting to note that the total eggshell calcium, but not the calcium concentration, was altered by DDT treatment.

Table 1. Eggshell Thickness, Weight and R-value from Control and DDT-Fed Ducks

Parameter	Control	50 ppm DDT*
Thickness (mm)	.412 ± .004	.337 ± .005
Weight (gm)	5.30 ± .11	4.68 ± .14
R-value**	.236 ± .004	.198 ± .006

* All three values are significant at $p < .01$.

** $R = \frac{\text{Weight}}{\text{Length} \times \text{Width}}$

NOTE: Each value represents a minimum of 19 eggshells ± 1 SD.

Table 2. Eggshell Weight, Calcium Concentration, and Total Calcium from Control and DDT-Fed Ducks

Measured value	Control	50 ppm DDT
gm		
Mean eggshell weight	5.50	4.26*
Mean Ca^{++}/gm eggshell	.384 ± .02	.371 ± .02
Mean total eggshell Ca^{++}	2.12 ± .29	1.57 ± .08*

* Significant at $p < .01$.

NOTE: Each group represents a minimum of six eggshells ± = 1 SD.

The ultrastructural composition of the representative microsomal pellets is shown in the figure. The principle organelles were rough endoplasmic reticulum, smooth endoplasmic reticulum, and fragments of microvilli and cilia.

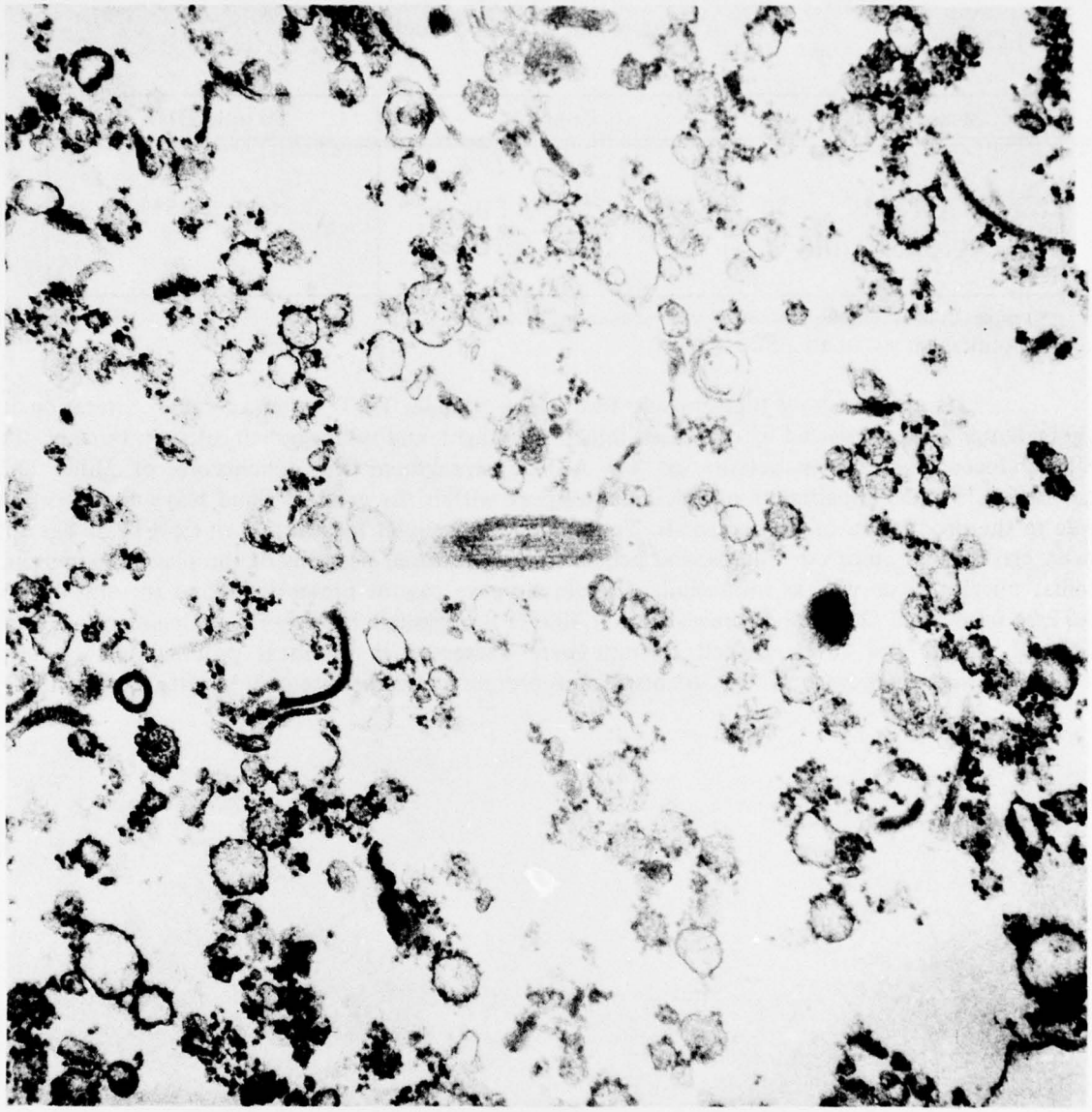

Figure. Ultrastructural Composition of Representative Microsomal Pellets

Specific activities of microsomal Ca ATPase from eggshell glands of control and DDT-fed ducks revealed a reduction of Ca ATPase ($P < .01$) after exposure (table 3). Enzyme activity in exposed ducks was 65% of control values.

Table 3. Microsomal Calcium ATPase Values from Eggshell of Control and DDT-Fed Ducks

Measured value	Control	50 ppm DDT
Ca ATPase*	9.75 ± 1.01	6.36 ± .66**
% Control activity	100	65

* μmoles Pi liberated/mg protein/30 min incubation.
** Significant at $p < .01 \pm = 1$ SD.

Our data show that chronic feeding of 50 ppm DDT to ducks causes alteration in eggshell quality as evidenced by decreased thickness, weight, and total eggshell calcium. Further, the DDT-induced decrease in activity of Ca ATPase strengthens the conclusions of Miller and coworkers,[10] that impairment of calcium transport within the eggshell gland plays an important role in the production of thin eggshells. To date, the subcellular localization of Ca ATPase has not been established. Since our microsomal pellets (figure) revealed portions of the plasma membrane (cilia, microvilli) as well as intracellular membranes, we cannot presently define the site of Ca ATPase inhibition. Our analyses revealed that 40% of the eggshell, by weight, is calcium. Since total eggshell weight and total eggshell calcium were decreased in identical proportions, we have concluded that other eggshell constituents such as protein and carbonate may be altered as well.

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